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# Early Season Population Dynamics and Residual Insecticide Effects on Bird Cherry-oat Aphid, Rhopalosiphum padi in Arkansas Winter Wheat

Beven McWilliams
*University of Arkansas, Fayetteville*

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# EARLY SEASON POPULATION DYNAMICS AND RESIDUAL INSECTICIDE EFFECTS ON BIRD CHERRY-OAT APHID, *RHOPALOSIPHUM PADI* IN ARKANSAS WINTER WHEAT

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By

Beven McWilliams
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## ABSTRACT

Bird cherry-oat aphid is a common pest of Arkansas winter wheat. This aphid vectors barley yellow dwarf virus which may cause extensive crop damage and yield loss when wheat is infested by virulent aphids in the fall. Some suggest this damage may be avoided using insecticide seed treatments if growers are unable to delay planting, as is recommended. Field population dynamics of bird cherry-oat aphid during fall 2009 and 2010 was assessed through random sampling of whole plants and pan trapping methods to evaluate aphid immigration. The field plots were divided into four subplots treated with a systemic insecticide seed treatment (thiamethoxam at the recommended rate of 0.148 liters of insecticide per 45 kilograms of seed or 5.0 fluid ounces per 100 lbs of seed) and four untreated plots. Aphids were counted twice weekly in ten 1 m row samples from plant emergence until the end of December. Aphids were classed as small or large nymphs and alates. Winged immigrants were also counted twice weekly in eight pan traps situated at equidistant points within the study fields. Aphid densities in untreated plots increased throughout the season and aphid densities were significantly lower in treated plots. In 2009 winged aphid numbers in untreated fields were significantly greater (mean = 6.54 per meter-row) than in the treated fields (mean = 0.03 per meter-row) ($t = 23.48$, $df = 639$, $P < 0.0001$). Large aphid numbers were greater in the untreated fields (mean = 5.17) than in the treated fields (mean = 0.43) ($t = 10.6$, $df = 639$, $P<0.0001$). Small aphid numbers were greater in the untreated fields (mean = 34.82) than in the treated fields (mean = 0.16) ($t = 19.88$, $df = 639$, $P<0.0001$). In 2010 winged aphid numbers were low but still differed between untreated fields (mean = 0.26 per meter-row) and treated fields (mean = 0.05 per meter-row) ($t = 8.75$, $df = 1197$, $P<0.0001$). Large aphid numbers were greater in the untreated fields (mean = 3.63 per meter-row) than in the treated fields (mean = 0.14 per meter-row) ($t = 15.82$, $df = 1198$, $P<0.0001$).

Small aphid numbers were greater in the untreated fields (mean = 34.82) than in the treated fields (mean = 0.16) ($t = 19.88$, $df = 639$, $P<0.0001$). Alate densities in the field were greatest early in the season, declining around two weeks after plant emergence. Winged aphids caught in pan traps roughly related to dates when winged aphids were observed in field samples. Greenhouse studies were conducted using potting soil as well as silt loam and clay (common soil types used for winter wheat in Arkansas). Trials conducted in the greenhouse showed that aphid numbers on treated plants were lower than untreated plants regardless of soil type for up to 20 days. However, insecticidal activity was longer in field-collected soils relative to potting soils, suggesting that future efficacy studies should use natural soils instead of potting soils. Based on field samples and greenhouse experiments, seed treated with thiamethoxam provided excellent control of immigrant aphids for up to 20 days. Thiamethoxam treatment of wheat seed provides some level of aphid suppression beyond the first 20 days after plant emergence, but additional studies are required to more accurately determine the duration of effective control.

This thesis is approved for recommendation
to the Graduate Council.

Thesis Director:

_____

Dr. Timothy Kring

Thesis Committee:

_____

Dr. Rick Cartwright

_____

Dr. Gus Lorenz

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# Introduction

## *Wheat*

Wheat (*Triticum* spp.) is a grass that was originally farmed in the Fertile Crescent region of the Near East. Wheat has been domestically cultivated since around 9,000 B.C. and is one of the most important cereals in the world (Briggle 1980). In 2010, the United States produced 1.49 billion bushels of wheat (NASS 2011). Wheat is a principal cereal grain used for human food and, in the United States, generally ranks fourth in agricultural production acreage of all field crops after corn, soybeans and hay (Ali 2002).

The wheat commercially grown in Arkansas is classified as soft red winter wheat, which is generally planted in late September through early November and harvested in June, and used primarily in the baking of cookies, cakes and related foods. This wheat has a soft endosperm, low protein content, and high yield potential (Briggle 1980), and is grown where winters are mild. Arkansas winter wheat was planted on 200,000 acres in 2010, producing 8.1 million bushels, or an average of 54 bushels per acre (NASS 2011).

Winter wheat planting and production are limited by climatic and biotic factors. Wheat production in the southern U.S. is also limited by damage from a variety of insect pests. The major pests or potential pests of wheat in Arkansas include Hessian fly, *Mayetola destructor* (Say) (Diptera: Cecidomyiidae) the true armyworm *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae), and several species of aphids (Homoptera: Aphidae) (Chapin et al. 2001).

## *Insect Pests*

The Hessian fly, *Mayetiola destructor* is a major insect pest of wheat grown in the southeastern U.S., especially if wheat is planted before the Hessian fly free date or in areas

where volunteer wheat is common. Hessian fly larvae feed on plant fluid at the base of the leaf sheath and can suppress growth of the plant if infested early (Cartwright et al. 1959). Up to 46% reduction in yield in winter wheat production has been recorded in Georgia (Buntin and Raymer 1989).

Armyworms feed on plant leaves, reducing the photosynthetic area of the plant, which has potential to reduce yield (Buntin 1986). Feeding by the true armyworm typically defoliates wheat in Arkansas after the grain is maturing, resulting in a recommendation to avoid treatment unless head-cutting is observed (Studebaker et al. 2011).

A different kind of plant feeding is that shown by aphids, which use specialized mouthparts to feed on plant phloem. Because of the systemic movement of fluids through phloem vessels in the plant, phloem-feeding pests, such as aphids, have the potential for vectoring various pathogens that can affect the entire plant (Irwin et al. 1988). Aphids feed on the plant phloem using fine stylets. The aphid excretes saliva to aid in feeding, which also may aid in transmitting plant viruses. Warm fall temperatures in the southern U.S. permit movement of aphids into winter wheat (Perry et al. 2000), thus aiding the potential for disease spread. Aphids that infest winter wheat in the southeastern United States, and that have the potential to vector diseases, include greenbug (*Schizaphis graminum* (Rondani)), English grain aphid (*Sitobion avenae* (F.)), corn leaf aphid (*Rhopalosiphum maidis* (F.)), and bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) (Flanders et al. 2006).

Greenbug is a bright green aphid with a dark stripe on the dorsal side, and clear cornicles that darken at the tip (Blackman and Eastop 2000). Greenbug lives on various cereals and grasses and may cause feeding damage from phytotoxins in the saliva. These aphids also have

the potential of vectoring diseases, such as barley yellow dwarf virus of the BYD-SGV subgroup (named after the primary aphid vector *Schizaphis graminum*).

English grain aphid is reddish-brown in color with black cornicles longer than the cauda (Blackman and Eastop 2000). This aphid feeds on upper leaves and cereal heads (Halbert and Voegtlin 1995). In South Carolina and other states, *S. avenae* is primarily a spring colonizer of winter wheat, with numbers reaching a peak around April or May (McPherson and Brann 1983, Chapin et al. 2001). This aphid can also transmit barley yellow dwarf virus of the BYDV-MAV and BYDV-PAV subgroups (named after the primary aphid vectors *Sitobion avenae* and *Rhopalosiphum padi* ).

Corn leaf aphid is yellow-green to dark olive-green with short, dark cornicles (Blackman and Eastop 2000). Corn leaf aphids that occur on winter wheat plants in the southeastern U.S. are at relatively low population levels in autumn (Halbert and Voegtlin 1995, Blackman and Eastop 2000, Chapin et al. 2001). The corn leaf aphid also transmits barley yellow dwarf virus of the BYDV-RMV subgroup (named after the primary aphid vector *Rhopalosiphum maidis*) (Power and Gray 1995).

The bird cherry-oat aphid is a widespread aphid found on wheat grown throughout the United States, including Arkansas. The adult aphid is about two millimeters long and usually olive-green with a reddish patch at the base of the cornicles. The aphid develops through four instars and molts four times before adulthood. Female adults of the bird cherry-oat aphid can be either winged (alate) or unwinged (apterous); the two states are known as alary dimorphism (Dixon 1985). Many factors can induce the development of wings, including overcrowding on the plant, inferior host quality, reduced day length, changes in temperature or any combination of these (Dixon 1985).

Many aphids, including bird cherry-oat aphid, alternate lineages of asexual and sexual reproduction cycles; the alteration is known as cyclical parthenogenesis. The bird cherry-oat aphid alternate from the primary host, *Prunus padus* (Rosales: Rosaceae), where the aphid reproduces sexually, to many cereals and grasses in the Poaceae, where the aphid reproduces asexually, allowing for rapid production of clones (Dixon and Glenn 1971, Dixon 1985). Cyclical parthenogenesis is common in temperate areas of the northern United States, where primary host plants are present and winter temperatures frequently drop below freezing. Aphids that alternate hosts reproduce sexually on the primary host, and lay eggs on that host. In climates with prolonged cold periods, eggs are the overwintering stage of the aphid (Robinson and Hsu 1963). In the southern United States, where winter temperatures are milder than in the north, aphids on cereals move lower on the plant, just below the soil surface, and overwinter as viviparous adults and nymphs (Chapin et al. 2001, Zwiener et al. 2005). Overwintering adult and nymphal aphids are able to tolerate short periods of temperatures below freezing by cold hardening (Zwiener et al. 2005).

The bird cherry-oat aphid is one of several aphid vectors of barley yellow dwarf virus (BYDV), particularly in the BYDV-PAV subgroup (named after the primary aphid vectors *Sitobion avenae* and *Rhopalosiphum padi* ) and BYDV-RPV (named after the primary aphid vector *Rhopalosiphum padi*) subgroup of the Cereal Yellow Dwarf Virus in the *Poleovirus* genus of the Leutoviridae family. Aphids feeding on plants infected by BYDV also aquire the virus (McKirdy and Jones 1997). Because these aphids are capable of vectoring BYDV, controlling aphid populations is the key to reducing the damage caused by BYDV in the field.

***Barley Yellow Dwarf Virus***

Barley yellow dwarf virus was first recognized in 1951 as the causal agent of yellow dwarf disease on barely in California (Oswald and Houston 1951). BYDV is in the Luteoviridae family and the sole member of the genus *Luteovirus* (Miller et al. 2002). The known host range of yellow dwarf viruses includes more than 150 species of the Poaceae including rice, corn and small grains (Gould and Shaw 1983). Annual and perennial grasses and volunteer cereals serve as reservoirs for virus, and therefore play an important role in BYDV epidemiology and transmission to cereal crops (Banks et al. 1995, El Yamani and Hill 1990).

Transmission of viruses by aphid vectors may be either non-persistent or persistent (Ossiannilsson 1966). Viruses like BYDV that are persistently transmitted require an aphid to feed directly in the phloem tissue, as opposed to just probing the surface, as is the case with non-persistent transmission. BYDV is transmitted only by aphids feeding on the phloem of the plant in which the virus is present (Miller and Rasochová 1997). Aphid probing to locate phloem tissue may take 20 to 30 minutes of feeding before a virus is ingested but, once the virus enters the aphid's body, the virus will persist through the aphid's life (Raman 1985).

The luteoviruses and subgroups are classified based on "stereological reaction" and specific vectors (Rochow 1979), as well as more-recent data on genome organization and sequencing and stereotype differences (Waterhouse et al. 1988). BYDV is caused by a group of phloem-limited luteoviruses transmitted by aphids circulatively, moving from the gut to salivary glands, and non-propagative in manner (Miller et al. 2002). Barley yellow dwarf virus was originally defined as: 1) a virus that is transmitted by aphids in a persistent manner, not mechanically; 2) does not replicate in the aphid but still circulates; 3) is not transmitted to offspring; 4) is confined to plant phloem tissue; and 5) has 25 nm icosahedral particles consisting

of a major ~22 kDa coat protein and a 52 kDa minor component encapsidating a 5.7- kb RNA (D'arcy et al. 2000). The subgroup dominant in Arkansas is BYDV-PAV, classified after the major vectors, *R. padi* and *S. avenae* (Miller & Rasochová 1997).

Symptoms of BYDV include yellowing and stunting of plants (Miller et al. 2002). BYDV can often go unrecognized because cereal agronomists may have trouble distinguishing symptoms caused by the virus from those symptoms caused by frost, wet weather, waterlogged soils, nutrition deficiencies, and other non-infectious factors (Conti et al. 1990), or other cereal diseases (Burnett 1990). Difficulties in disease recognition may lead to over- or under-rating the importance of the disease (Murray and Brown 1987). The presence of the virus can be evaluated by tests using BYDV-antibodies in enzyme-linked immunosorbent assay (ELISA) tests (Miller and Rasochová 1997, Zwiener et al. 2005).

***Barley Yellow Dwarf Virus and Bird Cherry-Oat Aphid Management***

Barley yellow dwarf virus is the most economically important virus of small grains and causes considerable damage and yield loss in crops throughout the world (Miller and Rasochová 1997, McKirdy and Jones 1997). Significant negative linear correlations between grain yield and BYDV incidence have been found (Smith and Sward 1982, Banks et al. 1995, McKirdy et al. 2002). Yield reductions of 34-67% from BYDV infection have been reported (Zwiener et al. 2005). BYDV can have a varying effect on yield depending on weather, the species of the vector, the virus subgroups, volunteer grasses and the plant stage (Pike 1990, Flanders et al. 2006). Aphid infestations in fields with BYDV during early plant growth stages in the fall may result in significant yield loss due to stunting of plants (Smith and Sward 1982, McKirdy and Jones 1996, Zwiener et al. 2005), and grain yields from fall infestations have been shown to be reduced an average of 63% (Cisar et al. 1982).

The pest status of bird cherry-oat aphid results from being the primary vector of BYDV. Feeding alone is not considered an issue. Aphid migration is important in BYDV spread (Irwin and Thresh 1990). Aphids flying into a field are immigrating aphids, which can come from a neighboring field or from miles away, if assisted by wind (Dixon 1998). Virulent aphids from another field can cause primary infections at scattered sites. Aphid immigration is common in the fall and in mild winters with warmer days that are adequate for aphid flight. Virulent aphids within a field cause secondary infection by wingless aphids moving to neighboring plants (Flanders et al. 2006). Aphids typically colonize wheat fields 1 to 2 weeks after plant emergence (Zwiener et al. 2005). Aphid populations on winter wheat generally increase slowly, if at all, through the mid-winter in the southern U.S. Increasing temperatures in the spring bring about an increase in aphid densities. Once the plant is no longer providing the nutrients needed for adequate growth toward the end of the growing season, or the plant is crowded, female aphids produce offspring that develop wings and emigrate to more suitable hosts within or among fields.

The recommended strategies for BYDV management include the cultural approach of late planting to limit fall colonization by aphids or chemical control using seed treatments and/or foliar application of insecticide to target aphids already present. The effectiveness of these strategies varies among regions (Bowen et al. 2002, Flanders et al. 2006).

Cultural control of aphids that transmit BYDV is the primary method of BYDV control in the southeast and in most temperate regions of the U.S. (Flanders et al. 2006). Control of immigrating aphids is possible by planting later in the season after the Hessian fly-free date or after the first hard freeze, which reduces aphid movement and reproduction. If fall flights are largely avoided, this results in lower aphid numbers through winter, preventing secondary infection (Flanders et al. 2006). Infection from viliferous aphids in the spring generally does not

cause yield loss of the magnitude associated with fall infection (Buntin and Chapin 1990). Although cultural control is considered highly effective, it is often difficult to implement this strategy, because of the short window of time between the Hessian-fly-free date and when weather in late fall prevents or delays planting.

When earlier planting dates are necessary due to weather or other agronomic factors, potentially viliferous aphids may appear in more fields at higher densities. Because management of BYDV relies on managing the aphid vectors of the disease, insecticides can be used to limit aphid colonization and to reduce the number of aphids that have already colonized the late-planted fields (Flanders et al. 2006). Treatment of wheat seed with effective insecticides can limit colonization by killing immigrating aphids before they reproduce or inoculate plants with BYDV. Foliar insecticide applications may reduce numbers of aphids, which can decrease the spread and prevalence of BYDV and, in turn, diminish yield loss (Gourmet et al. 1994, McKirdy and Jones 1996, Zwiener et al. 2005).

***Systemic Insecticides***

Many types of chemical control or insecticides are recommended for aphids in Arkansas winter wheat, including dimethoate, malathion, methomyl, and methyl parathion (Studebaker et al. 2011). Insecticides may be formulated as foliar sprays, soil drenches or seed treatments. Seed treatments metabolize and translocate within the plant tissue, although the duration of their effective control is limited. In contrast, foliar application of an insecticide has the potential of being washed off; not reaching the entire surface of the plant or the insecticide may be degraded by sunlight. Insecticides with systemic qualities (such as seed treatments) are thus considered most effective against aphids and other insects with a piercing and sucking mouth parts (Elbert et al. 1991).

One systemic insecticide used against aphids is the neonicotenoid, thiomethoxam (Flanders et al. 2006). Neonicotenoids are postsynaptic acetylcholine receptor agonists, in which pathways are blocked and acetylcholine accumulates (Tomizawa and Casida 2001). Thiamethoxam is a second-generation chlorothiazolymethyl neonicotenoid belonging to the thianicotinyl subclass (Maiensfisch et al. 2001). Thiamethoxam is marketed as a foliar, drench, soil, or seed treatment such as CuiserMaxx or Gaucho (Maiensfisch et al. 2001).

Neonicotenoids are highly effective against various insects including aphids, thrips, whiteflies, leaf miners, beetles and some lepidopteran species (Elbert et al. 1998, Maienfisch et al. 2001, Tomizawa and Casida 2005), and are generally used as systemic insecticides (Tomizawa and Casida 2003). These insecticides are often effective against insects that may be resistant to other classes of insecticides, such as pyrethroids, organophosphates, and carbamates (Denholm et al. 2002). Neonicotenoids are grouped together because of common structural similarities. These similarities can have the same effect on the insect and – if the insect becomes resistant to one, the insect may show cross-resistance to other insecticides in the same group based on metabolic mechanisms of the insecticide (Nauen and Denholm 2005, Prabhhaker et al. 2005).

Because of the potential for seed treatment to reduce risks associated with aphids colonizing winter wheat in the fall, I developed a project with the objective of assessing the benefits of seed treatment in winter wheat. In this project, I observed *R. padi* colonization in fields of winter wheat in Arkansas, and subsequent population changes. I also assessed the efficacy of a thiomethoxam seed treatment for its effects on aphid colonization and on those that had colonized wheat fields, as well as a greenhouse study that tested the residual activity effects of the insecticide in various soil types.

***Objectives of this study:***

1. To determine the immigration period of winged *R. padi* into winter wheat fields, using pan traps.
2. To observe population dynamics of *R. padi* in winter wheat fields in northwest Arkansas
3. To test the efficacy and persistence of thiamethoxam used as a seed treatment on aphids in the field as influenced by soil type.

## Materials and Methods

### *Alate aphid immigration*

Yellow pan traps (Kring 1972) were used to monitor immigration of aphids over two seasons (October through June) in 2009 and 2010 into two fields of soft red winter wheat at the Arkansas Agricultural Experiment Station in Fayetteville, Arkansas (the same fields used for the aphid immigration study). Pioneer 26R22 (Pioneer Hi-Bred International Inc., Johnston, Iowa) was used in the 2009-2010 growing season, planted on November 5, 2009. Pioneer 26R20 (Pioneer Hi-Bred International Inc., Johnston, Iowa) was used in year 2, planted on September 24, 2010. Pioneer 26R20 was used the second year because Pioneer 26R22 seed was unavailable. Pioneer 26R20 is a similar variety of wheat with resistance to diseases such as powdery mildew and stripe rust.

Pioneer 26R22 seeds were treated in the summer of 2009 and Pioneer 26R20 was treated during the summer of 2010 at the Lonoke Extension and Research Center near Lonoke, Arkansas. Seeds in both years were treated with thiamethoxam (Cruiser©, Syngenta Crop Protection, Greensboro, North Carolina), labeled for use in cereal grains, at the recommended rate of 0.148 liters of insecticide per 45 kilograms of seed (5.0 fluid ounces per 100 lbs of seed).

Two fields in close proximity to each other were used. The first field was 0.122 ha and the second field was 0.199 ha. The same fields were used each year, and were divided into eight smaller plots of alternating treated and untreated wheat. There were four replicates using a total of four treated plots and four untreated plots were monitored both the 2009-2010 and the 2010-2011 seasons. During the 2009-2010 season, the first field was divided into six plots of alternating treated and untreated seeds, and the second field was divided into two plots of treated

and untreated seeds. During the 2010-2011 season both fields were divided into four plots each (two treated, two untreated).

Yellow pan trap stands were constructed from 1.2m x 5cm x 5cm wooden stakes. Hardware cloth baskets 4cm x 20cm x 20cm were secured on the top, and one yellow plant saucer, 20 cm in diameter, was placed in each basket and secured with metal wire. Six yellow pan traps were uniformly placed in each of the two fields that were being sampled. These pans were painted bright yellow (Velspar® plastic paint code 68108) to attract aphids (Roach and Agee 1972), and placed ~1m above the ground. Traps were filled with a 70:30 mixture of water and propylene glycol to prevent freezing and desiccation. Pan traps were monitored every three days, all insects were removed and bird cherry-oat aphids counted. In 2009, pan traps were monitored from November 18 to December 20. In 2010, the monitoring occurred from October 4 until November 24.

***Aphid population dynamics***

Bird cherry-oat aphid densities were monitored in the same fields used for the aphid immigration study. Sampling for aphids in the field began November 18$^{th}$, (7 days after wheat emergence) and ended December 23$^{rd}$ for the 2009-2010 season. For the 2010-2011 season, aphid sampling began on October 4$^{th}$ (4 days after wheat emergence) and ended November 30th. Samples of aphids were taken twice weekly throughout the sampling period each year. Sampling dates were altered on occasion due to rainfall or snow cover. In each plot, for each sampling occasion, locations for ten random one-meter-row samples were identified using a 12-cm long, 7-mm diameter probe thrown into the plot. The landing point of the probe marked the center of the one-meter-row sample. Sampling consisted of counting all aphids on every plant in the one-

meter-row sample. All aphids were naturally occurring and were not removed or intentionally harmed when sampling. Bird cherry-oat aphids were classified as winged adults, large ($3^{rd}$- $4^{th}$ instars and apterous adult), or small ($1^{st}$ and $2^{nd}$ instars).

***Insecticide seed treatment residual activity***

A greenhouse study was conducted to determine the influence of soil type on the duration of efficacy of an insecticide applied to seeds. Wheat in Arkansas is commonly produced in both silt loam and clay soils, so both of these soils were evaluated. Calloway silt loam soil was obtained from fields near Lonoke, Arkansas, and Sharkey-Steele clay soil was obtained from fields near Keiser, Arkansas. Potting soil used as a control was Green Country Soil® (Miami, Oklahoma), containing compost, sand and perlite. Soil analysis was performed for each soil type at the University of Arkansas soil analysis lab to determine the initial levels of nutrients in the soil. All soils used were sieved and air dried in the greenhouse, then were infused with Expert Gardener Colorcote® slow-release, all-purpose plant food (11.97 ml fertilizer per 3.5 l of soil). Soil and fertilizer were mixed and potted for each soil type in 350 ml, 10-cm diameter terra cotta pots. Soil was reused in subsequent trials and fertilizer was reapplied after every third trial.

Each experiment used a total of 30 pots. Ten pots per soil type were filled to within 1cm of the rim of the pot. Five pots of each soil type were planted with seedlings of germinated Pioneer 26R20 seeds and five pots were planted with seedlings of the same cultivar germinated and treated with thiamethoxam as seeds at the recommended rate. All of the seeds were initially kept for four to five days at temperatures ranging from 20°C to 24°C in plastic dishes containing vermiculite, until germination occurred. Seedlings were used to eliminate the variation in germination time that was expected in the different soil types. Once emerged, seedlings that

were 2.5 cm in height were transferred to 10-cm diameter terra cotta pots and planted in the respective soil type. All plants were watered to maintain adequate soil moisture.

Bird cherry-oat aphid cultures were initiated each year from aphids collected from wheat fields in Washington County, Arkansas. Aphid cultures were kept on untreated young soft red winter wheat plants (Pioneer 26R20 cultivar) in cages in a greenhouse or inside a rearing building (22°C, 14:10 L:D). All aphid cages used in the experiment were 60 x 80 x 44 cm glass-top boxes constructed from plywood and painted white. Double-layer screen backs allowed air ventilation and excluded parasitoids and predators of the aphids. For each trial, ten fourth-instar *R. padi* were placed on each plant of the five treated or untreated plants in each soil type set.

The design was repeated for plants of different ages (4, 8, 10, 12, 14, 16, 18, 20, 26, and 48 days after seeds germinated). Each cage contained five treated or untreated plants of a given soil type. Cages with plants ages 4, 8, 10, 12, 18, 20 days since germination, were contained in a greenhouse at ambient temperature (<25° C max). However, after maximum temperatures in the greenhouse exceeded 25° C, the remaining experiments were conducted in cages inside a temperature-controlled indoor room, maintained at 22° C. The numbers of small, large and winged aphids were recorded daily to monitor mortality and natality. Counting continued daily until all of the aphids on the treated wheat in a cohort were dead or until the aphids on the untreated wheat reached numbers that caused overcrowding, and induced alate formation and/or visibly reduced the plant quality.

Plant size and age (days after emergence) were both expected to impact the concentration of the systemic insecticide in each plant. The width and length of every leaf of every plant were non-destructively measured (with a ruler) just before aphids were placed on the plants and again

when the trial ended. Length was measured from the bottom of each sheath to the tip of each leaf and width was measured at the widest part of the leaf. The sum of the leaf measurements was used as a crude estimate of leaf area to give an indication of plant size.

### *Statistical analysis*

Statistical analyses were conducted using JMP software with a predetermined alpha level set at $P < 0.05$ (SAS Institute 2010). One-way analyses of variance were performed on field samples and t-tests were used to detect significant differences between means of winged, small, and large aphids in treated and untreated fields during the two growing seasons. One-way analyses of variance were also performed on yield data and t-tests were used to detect significant differences between mean yields of treated and untreated fields after both growing seasons. Regression analyses were used to compare slopes of aphid densities over time on treated and untreated plants of clay, loam, and potting soil in greenhouse trials.

## Results

### *Alate aphid immigration 2009*

The first aphids found in pan traps (November 22) were caught 10 days after plant emergence. Winged aphids were collected in pan traps on three sample dates in 2009: 8 were collected on November 22, 9 on December 1 and 1 on December 21. Zero aphids were collected on four dates (November 20, 24, 27; December 4).

***Aphid population dynamics 2009***

Numbers of winged aphids were greater in the untreated fields (mean = 6.54 per meter-row) than in the treated fields (mean = 0.03 per meter-row) ($t = 23.48$, $df = 639$, $P < 0.0001$). Mean numbers sampled in untreated fields ranged from 4.6 per meter-row on November 18 to 10.0 on November 27, then declined over the last three sample dates to 1.4 on December 21 (Table 1a). In treated fields, mean numbers ranged from 0.0 to 0.05 through December 4, and then increased to a maximum of 0.15 on December 21 (Table 1a). On each of the eight sample dates, the mean numbers of winged aphids in the untreated fields were significantly greater than in the treated fields (Table 1a). There were significant interactions between treatment and replicate for three of the sample dates (Table 1a). Dead winged aphids were not counted but were commonly observed in treated fields, and less so in the untreated fields. Although dead aphids were observed, they were not counted because they persist across sampling dates and we assumed most would have dropped from the plant.

Numbers of large aphids were greater in the untreated fields (mean = 5.17) than in the treated fields (mean = 0.43) ($t = 10.6$, $df = 639$, $P<0.0001$). Mean numbers sampled in untreated fields increased from 0.0 per meter-row on November 18 to 17.7 on December 21 (Table 1b). In treated fields, mean numbers rapidly declined from a high of 2.9 on November 18 to 0.08 on November 18 and did not increase above 0.15 before sampling was terminated (Table 1b). On

six of the eight sample dates, the mean numbers in the untreated fields were significantly greater than in the treated fields (Table 1b). On November 18 (first sampling date), the numbers in the treated fields were significantly greater than the untreated fields (Table 1b). Two days later (November 20), the numbers did not differ significantly, whereas numbers in the untreated fields were significantly greater than in the treated fields on the final six sampling dates (Table 1b). There were significant interactions between treatment and replicate for five of the sample dates (Table 1b).

Numbers of small aphids were greater in the untreated fields (mean = 34.82) than in the treated fields (mean = 0.16) ($t = 19.88$, $df = 639$, $P<0.0001$). Mean numbers sampled in untreated fields increased from 5.5 per meter-row on Nov 18 to 79.7 on December 1, then declined on the final two sample dates (Table 1c). In treated fields, mean numbers declined from 1.1 on November 18 to zero or near-zero for the remaining sample dates (Table 1c). On all of the eight sample dates, the mean numbers in the untreated fields were significantly greater than in the treated fields (Table 1c). There were significant interactions between treatment and replicate for six of the sample dates (Table 1c).

Yields 2009. Wheat yields in treated fields averaged 55.5 bu/acre for the 2009 trials (harvested in June 2010), which was not significantly greater than the 47.1 bu/acre in the untreated fields ($t= 1.53$, $df = 7$, $P= 0.18$; Figure 1).

***Alate aphid immigration 2010***

The first winged aphids were collected in pan traps on October 4, 10 days after the planting date of September 24. Winged aphids were collected in pan traps on 11 sample dates in 2010: 4 aphids were collected on Oct 4, 7 on Oct 11, 3 on Oct 18, 2 on Nov 4, and 1 aphid was collected on 7 dates (October 15, 22, 25; November 1, 8,15, 22). Zero aphids were collected on three dates (October 29; November 12, 19).

***Aphid population dynamics 2010***

Numbers of winged aphids were low and did not differ between untreated fields (mean = 0.26 per meter-row) and treated fields (mean = 0.05 per meter-row) ($t = 8.75$, $df = 1197$, $P<0.0001$). Mean numbers sampled in untreated fields declined from 0.7 per meter-row on October 4, and remained fewer than 0.3 per meter-row throughout the season (except one sample of 0.55 on November 22) (Table 2a). In treated fields, mean numbers only exceeded 0.75 on one date (0.15 on October 14) (Table 2a). On 8 of the 15 sample dates, the mean numbers in the untreated fields were significantly greater than in the treated fields (Table 2a). There were significant interactions between treatment and replicate for one of the sample dates (Table 2a).

Numbers of large aphids were greater in the untreated fields (mean = 3.63 per meter-row) than in the treated fields (mean = 0.14 per meter-row) ($t = 15.82$, $df = 1198$, $P<0.0001$). Mean numbers sampled in untreated fields increased from 0.0 per meter-row on October 4 to 12.95 per meter-row on November 26 (Table 2b). In treated fields, mean numbers increased from 0.0 on October 14 to 0.75 on November 22, then declined to 0.68 on November 26 (Table 2b). On 11 of the 15 sample dates, the mean numbers in the untreated fields were significantly greater than

in the treated fields (Table 2b). There were significant interactions between treatment and replicate for eight of the sample dates (Table 2b).

Numbers of small aphids were greater in the untreated fields (mean = 3.01 per meter-row) than in the treated fields (mean = 0.16) ($t = 13.48$, $df = 1198$, $P<0.0001$). Mean numbers sampled in untreated fields increased from 0.42 per meter-row on October 4 to a high of 10.6 on November 22 (Table 1c). In treated fields, mean numbers ranged from 0.0 to 0.40 on all sample dates except November 22 and 26 (1.5 and 0.4, respectively) (Table 1c). Of 8 of the 15 sample dates, the mean numbers in the untreated fields were significantly greater than in the treated fields (Table 1c). There were significant interactions between treatment and replicate for six of the sample dates (Table 2c).

Yields 2010. Wheat yields in treated fields averaged 47.3 bu/acre for the 2010 trials (harvested in June 2011), which was not significantly greater than the 40.7 bu/acre in the untreated fields ($t= 1.22$, $df = 7$, $P= 0.27$; Figure 1).

***Insecticide seed treatment residual activity***

In general, for those treatments in which plants used were at least 14 days after germination, the numbers of aphids (combining small and large aphids) on untreated plants one day after placement on plants were slightly fewer than the initial number placed, regardless of soil type (Table 3). However, on treated plants, the numbers of aphids (large and small combined) one day after placement were fewer than half of the number placed on the plant one day earlier. Analyses showed that the slopes of aphid numbers over a seven-day period on untreated plants (slopes as measured by linear regressions) were all positive, whereas more than half of the slopes for treated plants were negative (Table 4).

For the trials in which aphids were placed on treated plants that were ages 4, 8, 10 and 12 days after germination, virtually all aphids were dead within 24 hours and none survived more than 4 days (Appendix 1). Aphid survival on untreated plants that were ages 4, 8, 10 and 12 days after germination was variable, with most numbers slightly increasing over the 2-4 day period (Appendix 1).

Clay Soil. The numbers of aphids alive after one day were greater on the untreated plants for four of the six treatments (days after germination). Only treatments 16 and 48 were not significant (Table 3). Changes in aphid numbers over a seven-day period after initial placement of aphids on treated plants, represented by slope of linear regressions, ranged from -0.24 aphids per day to +1.25 aphids per day. Treatments 14 and 18 days since germination were the only ones with slopes significantly different from zero, and both slopes were negative. For untreated plants, slopes ranged from 0.41 aphids per day to 11.33 aphids per day. Growth of aphids on plants that were 14, 16, 18 and 20 days after germination had significant positive slopes, whereas slopes were not significantly different from zero for treatments 26 and 48 days since germinations (Table 4).

Loam Soil. The numbers of aphids alive after one day were greater on the untreated plants for five of the six treatments (days after germination). Only the treatment 48 days after germination resulted in no significant differences between treated and untreated numbers (Table 3). Changes in aphid numbers over a seven-day period after initial placement of aphids on treated plants, represented by slope of linear regressions, ranged from -0.78 aphids per day to +1.19 aphids per day. Treatments 14, 18 and 20 days since germination were the only slopes significantly different from zero. The slope for the treatment 14 days was positive, whereas the slopes for the treatments 18 and 20 days were negative (Table 4). Slopes for the treatments 16, 26 and 48 days

after germination did not differ from zero. For untreated plants, slopes ranged from 0.07 aphids per day to 7.37 aphids per day. Growth of aphids on plants that were 14, 16, 20 and 26 days after germination had significant positive slopes, whereas slopes were not significantly different from zero for the treatments 18 and 48 days since germinations (Table 4).

Potting Soil. The numbers of aphids alive after one day were greater on the untreated plants for four of the six treatments (days after germination). Only the treatments 26 and 48 days after germination were not significantly different from numbers of aphids on untreated plants (Table 3). Changes in aphid numbers over a seven-day period after initial placement of aphids on treated plants, represented by slope of linear regressions, ranged from -0.41 aphids per day to +2.48 aphids per day. The treatments 14 and 26 days after germination had positive slopes significantly different from zero (Table 4). The slopes for treatments 16, 18, 20 and 48 days after did not differ from zero. For untreated plants, slopes ranged from 0.19 aphids per day to 9.11 aphids per day. The treatments 14, 16, 18 and 20 days since germination had significantly positively slopes. The slopes for the treatments 26 and 48 after germination did not differ from zero (Table 4).

## Discussion

***Aphid population dynamics 2009*** Winged aphids collected in pan traps roughly corresponded to dates when they were collected in the field samples – this correspondence implies that winged aphids were arriving from elsewhere, as opposed to production of winged aphids in the field. Also, numbers never reached a density at which we would expect winged aphids would develop. Rapid insecticidal activity was evidenced by the significant reduction in the number of winged aphids in treated fields. In 2009 untreated fields, numbers of aphids averaged fewer than 10 per meter-row for the entire sampling period (November 18 – December 21). In the treated plots, numbers of winged aphids reached a maximum of 0.15 per row-meter – at the end of the fall season (December 21). Although the duration of the efficacy of the seed treatment is not known under conditions in Arkansas, the small numbers of winged adults in treated fields early in the season resulted in no production of offspring throughout the season, thus averting any population growth. A lack of aphids in the treated plots also corresponded to a lack of vectors for BYDV, if present. However, despite the efficacy in reducing numbers of all stages of aphids, wheat yields in the treated fields were not greater than in the untreated fields of the experiment. This was expected as yield loss due to bird cherry-oat aphid feeding alone has rarely been documented (Leather and Dixon 1981, Saheed et al. 2007). Therefore, the added cost of the seed treatment – while effective at killing aphids – was not returned in significantly greater crop yields.

### *Aphid population dynamics 2010*

Although the aphid numbers in field samples and pan-trapping in 2010 were greatly reduced, sampling was likely more effective due to increased plot sizes. Larger plots permitted sampling to cause less damage to the overall plots and aphids on the plants.

In 2010, wheat was planted about a month earlier. Again, there were differences in numbers of aphids in treated versus untreated fields, although fewer differences in winged adults and the pattern of differences were not as consistent. The overall numbers of winged aphids in the field samples in 2010 were much smaller in both treated and untreated fields than those sampled in 2009, which likely affected the patterns seen. Although delayed planting is recommended to avoid earlier aphid flights (Royer et al. 2005), annual weather differences result in variable arrival of immigrating aphid flights. Numbers of small and large aphids increased mid-season (October 25) in the untreated fields (Table 2a), whereas no increases were seen in the treated fields until November 22. Again, the lack of adults in the treated fields precluded any production of offspring until late in the season and despite the efficacy in reducing numbers of all stages of aphids, wheat yields in the treated fields were not significantly greater than in the untreated fields of the experiment.

### *Insecticide seed treatment residual activity*

The greenhouse trials showed that the seed treatment killed aphids effectively. On small, seed-treated plants (4, 8, 10 and 12 days after germination), nearly all aphids were dead within one day. Those few that survived on treated plants were all dead within 4 days (these trials were discontinued because all aphids on treated plants were dead). Because aphids on treated plants only lived for 4 days or less, analysis of differences was not considered meaningful, partly

because of the small sizes of the plants, and the short duration of the trials. This consistent activity in the first days of the trial confirmed the efficacy of these seed treatments immediately after plant emergence. Other studies have shown Cruiser© is very effective immediately after plant emergence (Magalhaes et al. 2008).

Survival after 24-h on treated and untreated plants that were tested 48 days after germination was not significantly different. Although the numbers of aphids on treated plants after 24 hours were small, the numbers on untreated plants were the smallest of any of the trials; thus, there was no difference in 24-hour survival between treated and untreated plants. Nor were there any consistent patterns for differences in slopes for increases in aphid numbers over seven-day periods among the three soil types. Numbers of aphids on both treated and untreated plants remained low over the 7 days the trials were observed, not exceeding 10 aphids per plant. Because the trials were conducted on plants grown in the greenhouse, the sizes of plants in the 48-day-after germination trials were much larger than would be experienced in the field – those plant sizes may not have occurred until spring (depending on fall temperatures and precipitation).

For the plants that were used 26 days after germination, 24-hour survival was greater on untreated plants in both clay and loam soils, but not different on untreated plants grown in potting soil. However, the slopes of aphid numbers over seven-day periods for treated plants in both clay and loam were negative, whereas the slope for aphid growth on treated plants in potting soil was positive. Aphid numbers on treated plants of this age never reached high values, as the slopes of aphid numbers over the seven-day periods were lower than for untreated plants in all trials with younger plants. The marked difference in the results from plants grown in potting soil relative to those from field soils suggests that use of potting soils for efficacy

evaluation of seed treatments may yield different results than might be observed in the field. Therefore, we recommend the use of field soils for all seed treatment evaluations.

The greenhouse trials on plants that had germinated at least 14 days but less than 26 days before placement showed that, regardless of soil type and number of days after germination, the seed treatment was effective at killing most – but not all – aphids within 24 hours after being introduced to the treated plants. The few that survived increased in number, but at a low rate – in some trials – but decreased to zero in others. This depressed reproduction may result from a sublethal effect on the aphids by the insecticides. In contrast, aphid numbers on untreated plants increased, in some trials, to an average of more than 100 aphids per plant after 7-9 days. Therefore, the growth of aphid numbers in untreated plants, coupled with greater 24-hour survival, showed that the seed treatment was effective at least for 20 days after germination under greenhouse conditions.

Although the ages after germination of the treatments in field experiments illustrated the duration of the seed treatment in the plants, those ages did not accurately reflect differences in plant sizes. For example, plants in the 14-day trials were larger than plants that received aphids 16 and 18 days after germination. If the concentration of insecticide in the plant is affected by plant size – i.e., total leaf area – then larger plants of a similar-aged plant may have less insecticide, thus affecting aphid survival. Controlling for plant size for each age could have reduced some of the variability seen in aphid survival and growth in numbers.

In all trials, if aphid numbers on treated plants increased, those increases came after at least a week since placement on the plants. Increases in numbers of aphids in late fall would occur at a time when temperatures are generally lower. As a result of lower temperatures, aphid

flight (immigrating aphids only) would be limited to warm days only. The combination of few aphids, cool temperatures, and limited aphid flight would lead to low probability of disease transmission. Therefore, in northwest Arkansas the seed treatment was effective long enough to kill immigrating aphids while temperatures would still be warm. Further, the duration of the seed treatment was sufficient to keep founding aphid numbers low enough to suppress aphid population growth, therefore preventing densities that would result in aphid movement and – again – reducing probability of disease transmission.

Although the seed treatment was effective at keeping aphid numbers low on treated plants, there were no statistically significant differences in yield between treated and untreated plants in this study. However, the numbers of aphids in the field were low – the numbers may have needed to be much greater to detect impact on the plant and subsequent yield differences. In the field trials, numbers of aphids only once reached a level of 80 per meter-row. In Arkansas, treatment of bird cherry-oat aphid populations is not currently recommended (Studebaker et al. 2011). The low numbers of aphids found in these trials may not have provided the definitive answer on the cost-effectiveness of the seed treatment if one were evaluating the impact of aphid feeding alone. In areas in which BYDV occurs only infrequently, seed treatment to prevent loss in the infrequent years may not be economically viable, even though the seed treatment killed aphids. In areas in which BYDV occurs regularly or reliably, the efficacy of the seed treatment may result in economic benefit. In order to determine utility and economic benefit of seed treatment, a grower must consider the likelihood of BYDV occurring, planting date, aphid flights, total input costs and the price of wheat.

## Future Work

The first year was planted late in the season and the second year was planted earlier. One of the interesting observations was the difference in aphid populations that may be due to aphid colonization to young plants and population growth on plants of different ages. Observation of plants in the same area planted at different times at different stages of and observing the population dynamics could provide more robust results.

The greenhouse study demonstrated that the insecticide efficacy was lost between 26 and 48 days after emergence. Future trials could focus on this period and eliminate trials less than 21 days after plant emergence.

The small pots used in the greenhouse may have a different effect on the draining of the insecticide in the soil versus the field. Insecticide washed into the soil may be diluted and moved differently in the soil and field experiments, thus having an impact on the residual effects of the insecticide. Insecticide retention in pots may have increased the duration of insecticide efficacy because the insecticide may not have diluted at the same rate as it would in the field. Alternatively, increased drainage due to the drainage hole in the pot may have reduced the impact of the insecticide. Deeper pots or trays might mimic the movement of seed treatment deeper in the soil.

The pan traps could be put up earlier to detect aphid flight before field sampling. This would give a better profile of aphid flight during the season and a starting point of aphid movement. The pan traps may also not have been optimal for trapping aphids to assess densities (Fereres et al. 1999). Optimal yellow color with colorfast traps to prevent trap color fading in the sun could stop the attractiveness change over time in the field.

Wheat in Arkansas is grown primarily on variations of loam soil. Potting soil and clay were chosen for a broad array of soils from optimal (potting soil) to less optimal. All of the potted plants had the same amount of fertilizer added. Despite the differences in soil nutrition among the soil types, there was no great difference in 24 hour survival as a function of soil types. Concentrating future studies on one typical field soil (i.e., loam) would permit testing greater numbers of plants per trial. The atypical responses in potting soil indicate that field soils should be used for testing pesticide seed treatments.

## Tables

Table 1. Mean numbers (plus variance) of winged aphids (1a), large aphids (1b) and small aphids (1c) collected from ten, 1 meter-row whole plant samples in the field during 2009. Comparisons were made between numbers of aphids on seed-treated plants and untreated plants. Large aphids included 3rd-4th –instars and alate adults. Small aphids were 1st and 2n instars. Tests were performed using SAS (SAS Institute Inc. 2010).

1a.

| | **Treated** | **Untreated** | **Probability > F** | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **11/18** | 0.05 ± (0.05) | 4.55 ± (12.2) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/20** | 0.00 ± (0.00) | 7.15 ± (14.01) | 0.16 | <0.0001 ŧ |
| **11/22** | 0.00 ± (0.00) | 6.70 ± (12.37) | 0.64 | <0.0001 ŧ |
| **11/25** | 0.00 ± (0.00) | 11.15 ± (12.75) | 0.08 | <0.0001 ŧ |
| **11/27** | 0.00 ± (0.00) | 10.02 ± (54.07) | <0.0001 ŧ | <0.0001 ŧ* |
| **12/01** | 0.03 ± (0.03) | 7.63 ± (4.91) | 0.66 | <0.0001 ŧ |
| **12/04** | 0.00 ± (0.0) | 3.63 ± (10.65) | 0.0006 ŧ | <0.0001 ŧ* |
| **12/21** | 0.15 ± (0.18) | 1.38 ± (2.39) | 0.11 | <0.0001 ŧ |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

1b.

| | **Treated** | **Untreated** | **Probability > F** | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **11/18** | 2.93 ± (15.10) | 0.00 ± (0.00) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/20** | 0.08 ± (0.13) | 0.00 ± (0.00) | 0.54 | 0.18 |
| **11/22** | 0.15 ± (0.18) | 0.95 ± (3.18) | 0.18 | 0.006 ŧ |
| **11/25** | 0.10 ± (0.14) | 4.20 ± (9.50) | 0.01 ŧ | <0.0001 ŧ* |
| **11/27** | 0.10 ± (0.09) | 5.07 ± (22.22) | 0.01 ŧ | <0.0001 ŧ* |
| **12/01** | 0.00 ± (0.00) | 6.25 ± (5.37) | 0.16 | <0.0001 ŧ |
| **12/04** | 0.02 ± (0.03) | 7.35 ± (45.32) | <0.0001 ŧ | <0.0001 ŧ* |
| **12/21** | 0.02 ± (0.03) | 17.68 ± (175.4) | <0.0001 ŧ | <0.0001 ŧ* |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

1c.

| | **Treated** | **Untreated** | **Probability > F** | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **11/18** | 1.08 ± (2.17) | 5.53 ± (30.46) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/20** | 0.03 ± (0.03) | 20.88 ± (161.41) | 0.04 ŧ | <0.0001 ŧ* |
| **11/22** | 0.05 ± (0.05) | 42.28 ± (451.33) | 0.09 | <0.0001 ŧ |
| **11/25** | 0.03 ± (0.03) | 50.15 ± (284.54) | 0.07 | <0.0001 ŧ |
| **11/27** | 0.13 ± (0.11) | 48.71 ± (1175.51) | 0.001 ŧ | <0.0001 ŧ* |
| **12/01** | 0.00 ± (0.00) | 79.65 ± (792.28) | 0.001 ŧ | <0.0001 ŧ* |
| **12/04** | 0.00 ± (0.00) | 25.28 ± (476.31) | <0.0001 ŧ | <0.0001 ŧ* |
| **12/21** | 0.00 ± (0.00) | 6.08 ± (17.76) | <0.0001 ŧ | <0.0001 ŧ* |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

Table 2. Mean numbers (plus variance) of winged aphids (1a), large aphids (1b) and small aphids (1c) collected from ten, 1 meter-row whole plant samples in the field during 2010. Comparisons were made between numbers of aphids on seed-treated plants and untreated plants. Large aphids included 3rd, 4th –instars and alate adults. Small aphids were 1st and 2nd instars. Tests were performed using an effect test (SAS Institute Inc. 2010).

2a.

| | **Treated** | **Untreated** | **Probability > F** | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **10/04** | 0.08 ± (0.07) | 0.71 ± (0.76) | 0.26 | 0.006 ŧ |
| **10/11** | 0.08 ± (0.07) | 0.68 ± (0.53) | 0.44 | 0.06 |
| **10/14** | 0.15 ± (0.18) | 0.53 ± (0.31) | 0.03ŧ | 0.007 ŧ* |
| **10/18** | 0.08 ± (0.07) | 0.30 ± (0.27) | 0.36 | 0.16 |
| **10/21** | 0.00 ± (0.00) | 0.20 ± (0.22) | 0.41 | 0.05 |
| **10/25** | 0.00 ± (0.00) | 0.10 ± (0.09) | 0.01 ŧ | <0.0001 ŧ* |
| **10/28** | 0.00 ± (0.00) | 0.13 ± (0.11) | 0.13 | <0.0001 ŧ |
| **11/01** | 0.00 ± (0.00) | 0.08 ± (0.07) | 0.36 | <0.0001 ŧ |
| **11/04** | 0.05 ± (0.05) | 0.00 ± (0.00) | 0.0005 ŧ | <0.0001 ŧ* |
| **11/08** | 0.03 ± (0.03) | 0.08 ± (0.07) | 0.01 ŧ | <0.0001 ŧ |
| **11/11** | 0.08 ± (0.07) | 0.20 ± (0.22) | 0.003 ŧ | <0.0001 ŧ* |
| **11/15** | 0.08 ± (0.07) | 0.25 ± (0.29) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/18** | 0.00 ± (0.00) | 0.05 ± (0.05) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/22** | 0.05 ± (0.05) | 0.55 ± (0.97) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/26** | 0.05 ± (0.05) | 0.10 ± (0.14) | 0.73 | <0.0001 ŧ |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

2b.

| | Treated | Untreated | Probability > F | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **10/04** | 0.00 ± (0.00) | 0.00 ± (0.00) | · | · |
| **10/11** | 0.00 ± (0.00) | 0.15 ± (0.34) | 0.45 | 0.11 |
| **10/14** | 0.00 ± (0.00) | 0.00 ± (0.00) | · | · |
| **10/18** | 0.00 ± (0.00) | 0.13 ± (0.13) | 0.89 | 0.03 ŧ |
| **10/21** | 0.15 ± (0.90) | 0.13 ± (0.13) | 0.79 | 0.88 |
| **10/25** | 0.00 ± (0.00) | 0.80 ± (1.81) | 0.001 ŧ | <0.0001 ŧ* |
| **10/28** | 0.05 ± (0.05) | 1.73 ± (7.18) | 0.13 | 0.0001 ŧ |
| **11/01** | 0.00 ± (0.00) | 2.40 ± (3.27) | 0.46 | <0.0001 ŧ |
| **11/04** | 0.03 ± (0.03) | 3.28 ± (0.51) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/08** | 0.03 ± (0.03) | 5.65 ± (25.92) | 0.10 | <0.0001 ŧ |
| **11/11** | 0.10 ± (0.09) | 6.08 ± (15.51) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/15** | 0.20 ± (0.27) | 7.75 ± (19.47) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/18** | 0.05 ± (0.04) | 7.27 ± (10.68) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/22** | 0.75 ± (1.78) | 6.23 ± (53.92) | <0.0001 ŧ | <0.0001 ŧ* |
| **11/26** | 0.68 ± (1.10) | 13.0 ± (80.77) | 0.22 | <0.0001 ŧ |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

2c.

| | **Treated** | **Untreated** | **Probability > F** | |
|---|---|---|---|---|
| **Date** | Mean ± Variance | Mean ± Variance | Rep | Treatment |
| **10/04** | 0.00 ± (0.00) | 0.41 ± (0.90) | 0.91 | <0.0001ŧ |
| **10/11** | 0.00 ± (0.00) | 0.23 ± (0.54) | 0.89 | <0.0001ŧ |
| **10/14** | 0.00 ± (0.00) | 0.38 ± (0.86) | 0.37 | 0.0008ŧ |
| **10/18** | 0.08 ± (0.02) | 0.45 ± (2.61) | 0.09 | 0.01ŧ |
| **10/21** | 0.00 ± (0.00) | 0.13 ± (0.16) | 0.12 | 0.006ŧ |
| **10/25** | 0.03 ± (0.03) | 0.95 ± (1.89) | 0.55 | 0.04ŧ |
| **10/28** | 0.00 ± (0.00) | 1.68 ± (6.79) | 0.10 | 0.02ŧ |
| **11/01** | 0.03 ± (0.03) | 2.50 ± (3.69) | 0.27 | 0.08 |
| **11/04** | 0.03 ± (0.03) | 2.43 ± (5.69) | 0.09 | 0.14 |
| **11/08** | 0.00 ± (0.00) | 2.83 ± (6.20) | 0.16 | 0.31 |
| **11/11** | 0.00 ± (0.00) | 4.50 ± (16.05) | 0.06 | 0.13 |
| **11/15** | 0.30 ± (1.04) | 6.28 ± (16.00) | 0.50 | 0.07 |
| **11/18** | 0.08 ± (0.12) | 6.28 ± (15.18) | 0.58 | 0.16 |
| **11/22** | 1.53 ± (8.46) | 10.63 ± (151.50) | <0.0001ŧ | <0.0001ŧ* |
| **11/26** | 0.40 ± (1.37) | 5.55 ± (36.20) | 0.45 | 0.47 |

1. ŧ Indicates significant treatment effect.
2. * Indicates significant interaction between treatment and rep (P<0.05).

Table 3. Direct counts of aphids on wheat plants grown in different soils, indicating aphid survival 24 hours after the initial aphid application of ten large aphids per plant totaling 50 aphids per soil-type and treatment by day (including $4^{th}$ –instars and alate adults). Clay loam and potting soils for every trial were compared between treated and untreated plants treated as seeds. Treatments consisted of plants of different ages since germination. Tests were performed using a t-test (SAS Institute Inc. 2010).

3a.

| **Clay** | | | |
|---|---|---|---|
| **Day** | Treated | Untreated | P-Value |
| **14** | 8 | 55 | 0.003* |
| **16** | 19 | 32 | 0.151 |
| **18** | 9 | 48 | 0.001* |
| **20** | 33 | 48 | 0.001* |
| **26** | 18 | 52 | 0.004* |
| **48** | 18 | 19 | 0.424 |

3b.

| **Loam** | | | |
|---|---|---|---|
| **Day** | Treated | Untreated | P-Value |
| **14** | 13 | 54 | 0.016* |
| **16** | 16 | 45 | 0.001* |
| **18** | 16 | 44 | 0.004* |
| **20** | 41 | 55 | 0.004* |
| **26** | 11 | 34 | 0.013* |
| **48** | 14 | 21 | 0.128 |

3c.

| **Potting** | | | |
|---|---|---|---|
| **Day** | Treated | Untreated | P-Value |
| **14** | 13 | 50 | 0.022* |
| **16** | 23 | 57 | 0.005* |
| **18** | 14 | 45 | 0.008* |
| **20** | 32 | 62 | 0.008* |
| **26** | 17 | 36 | 0.112 |
| **48** | 8 | 25 | 0.567 |

1. * Indicates significance difference between treatments

Table 4. Slope of population growth of *R. padi* on wheat plants grown in different soils. Linear slopes were calculated for 7-day periods after aphids were placed on plans. Treatments consisted of plants of different ages since germination. Slopes were compared to determine differences from zero. Tests were performed using a regression analysis (SAS Institute Inc. 2010). Slopes were not calculated for plants aged 4, 8, 10, and 12 days, due to short duration of survival.

4a.

| **Clay** | | | | |
|---|---|---|---|---|
| **Day** | Treated | P-Value | Untreated | P-Value |
| **14** | -0.18 | 0.014 ŧ | 10.51 | 0.0001 ŧ |
| **16** | 0.49 | 0.376 | 6.75 | 0.0001 ŧ |
| **18** | -0.22 | 0.008 ŧ | 3.51 | 0.0003 ŧ |
| **20** | 1.25 | 0.131 | 11.33 | 0.0001 ŧ |
| **26** | 0.24 | 0.146 | -0.94 | 0.0125 ŧ |
| **48** | 0.16 | 0.308 | 0.41 | 0.136 |

4b.

| **Loam** | | | | |
|---|---|---|---|---|
| **Day** | Treated | P-Value | Untreated | P-Value |
| **14** | 1.19 | 0.045 ŧ | 7.37 | 0.0001 ŧ |
| **16** | 0.71 | 0.177 | 7.32 | 0.0001 ŧ |
| **18** | -0.36 | 0.003 ŧ | 1.29 | 0.271 |
| **20** | -0.78 | 0.0001 ŧ | 5.01 | 0.0001 ŧ |
| **26** | -0.30 | 0.049 ŧ | -1.43 | 0.0001 ŧ |
| **48** | -0.03 | 0.9102 | 0.07 | 0.855 |

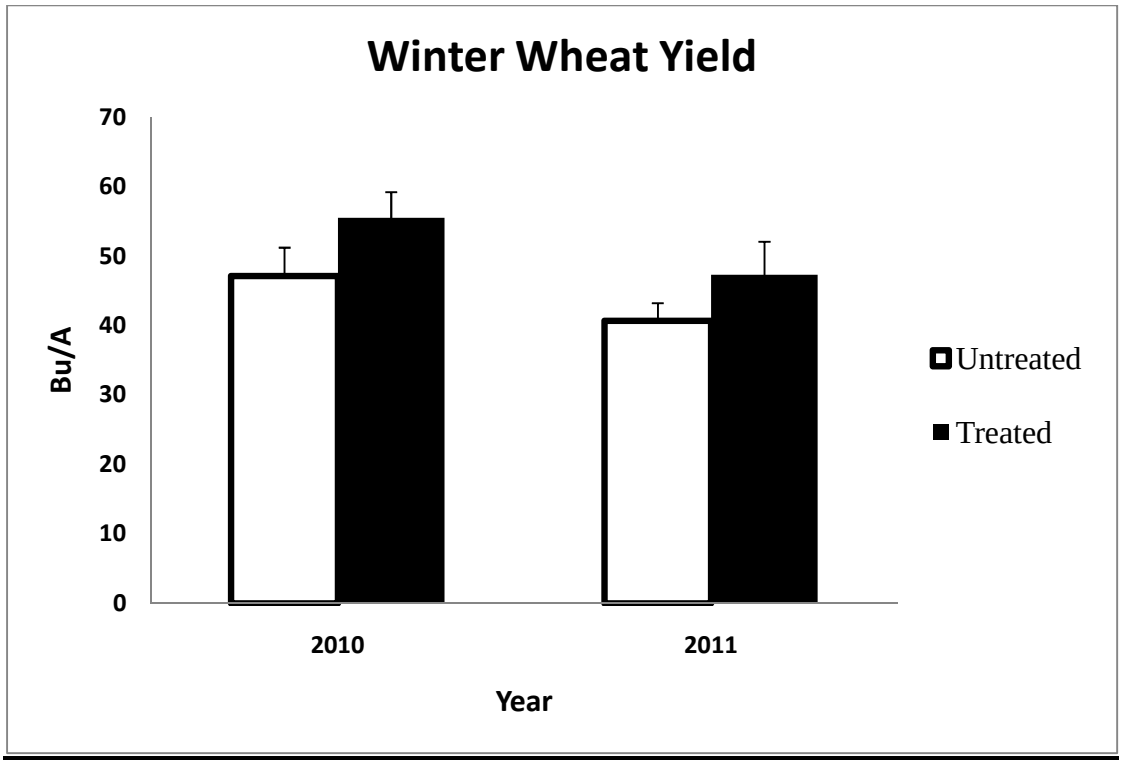
4c.

| **Potting** | | | | |
|---|---|---|---|---|
| **Day** | Treated | P-Value | Untreated | P-Value |
| **14** | 2.48 | 0.031 ŧ | 9.11 | 0.0001 ŧ |
| **16** | 0.89 | 0.136 | 8.81 | 0.0001 ŧ |
| **18** | -0.20 | 0.646 | 6.43 | 0.0001 ŧ |
| **20** | -0.41 | 0.135 | 4.09 | 0.0001 ŧ |
| **26** | 2.73 | 0.0001 ŧ | -1.25 | 0.024 ŧ |
| **48** | -0.05 | 0.862 | 0.55 | 0.166 |

1. ŧ Indicates significance from zero.

## Figures

Figure 1. Yield data taken from Arkansas university experiment station field plots during the 2009 and 2010 field seasons.



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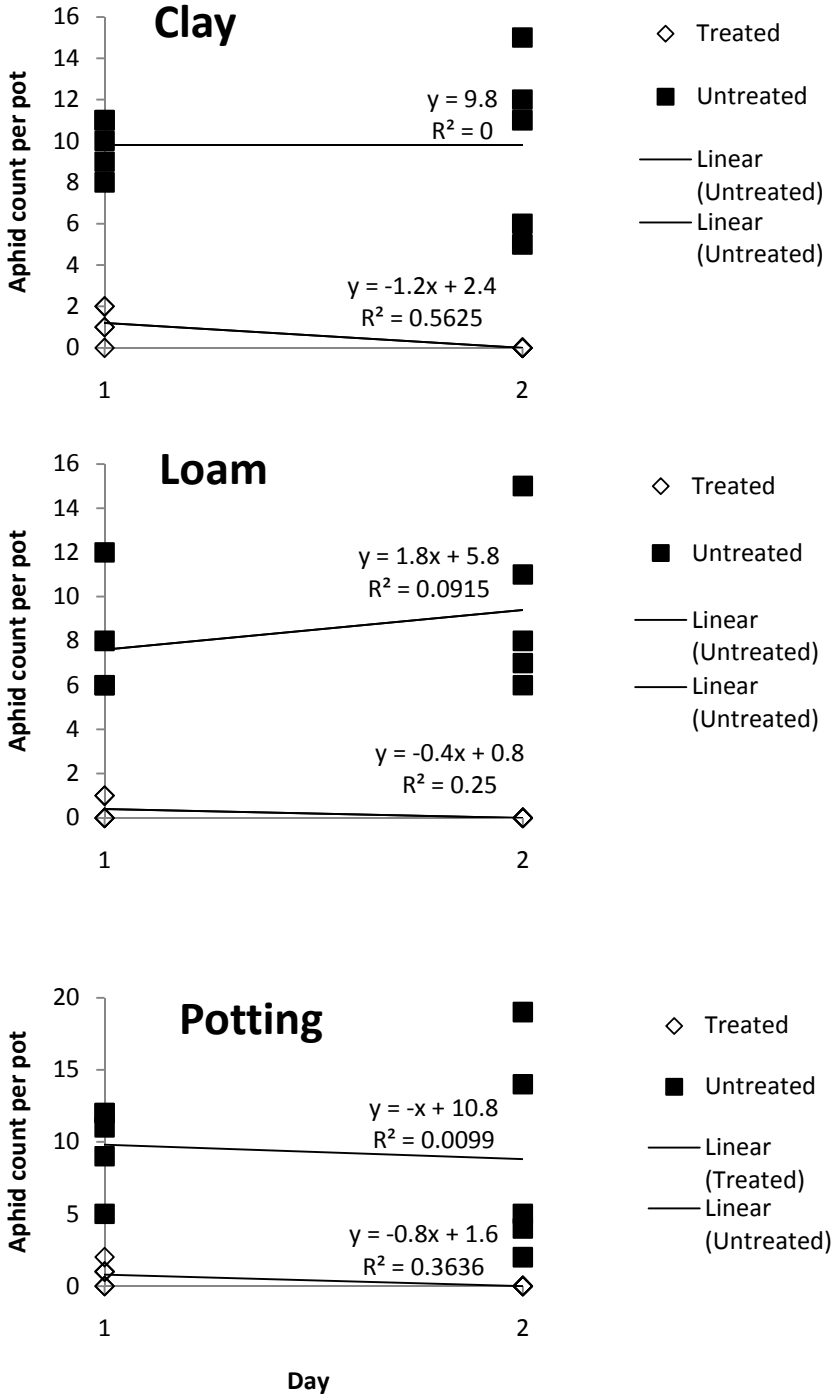
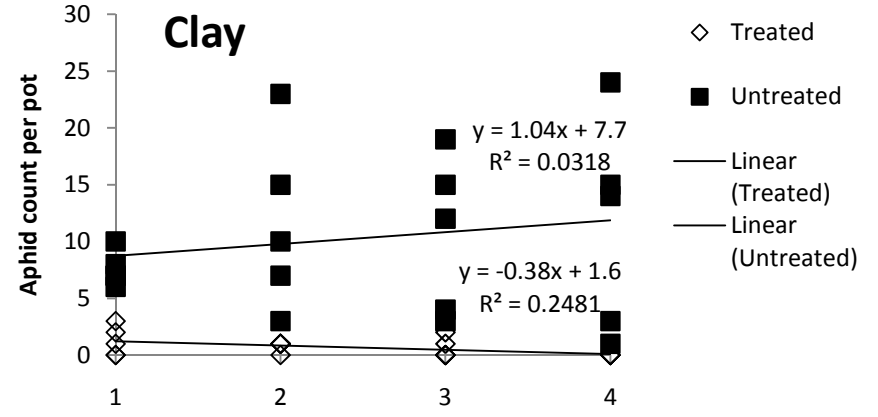
## Appendix

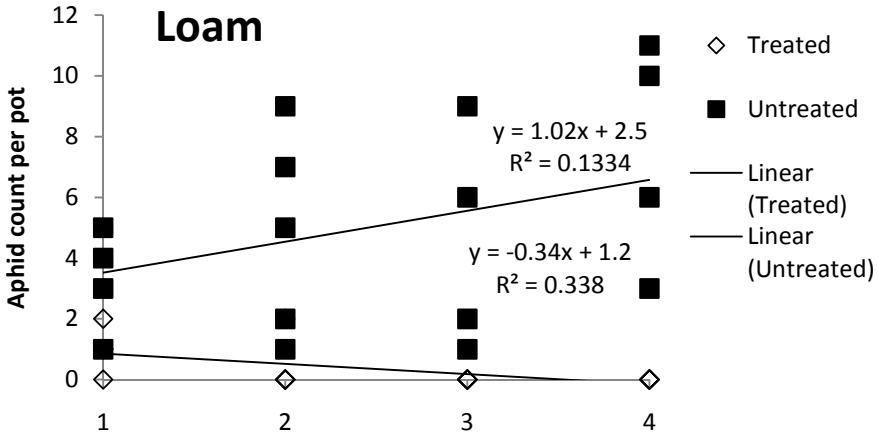
1. Aphid survival on untreated plants ages 4 (1a.), 8(1b.), 10(1c.) and 12(1d.) by day for clay loam and potting soil greenhouse experiments.

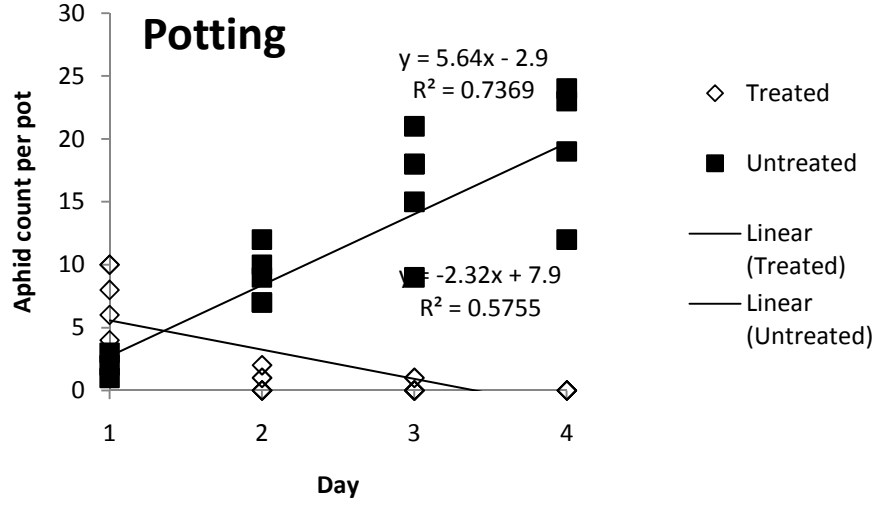
1a. Day 4.



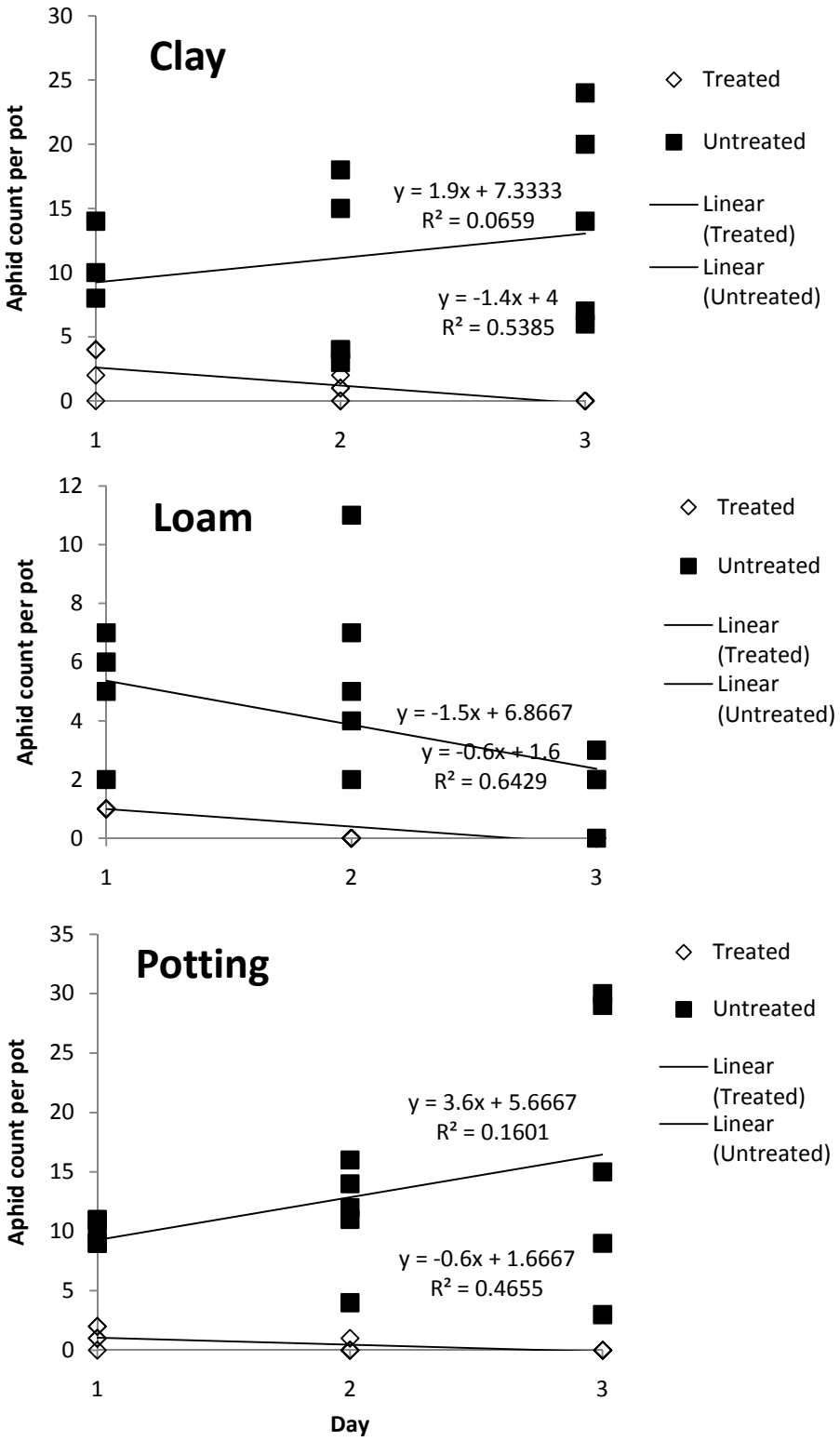
1b. Day 8.


Clay
Aphid count per pot
y = 1.04x + 7.7
R² = 0.0318
y = -0.38x + 1.6
R² = 0.2481
Treated
Untreated
Linear (Treated)
Linear (Untreated)

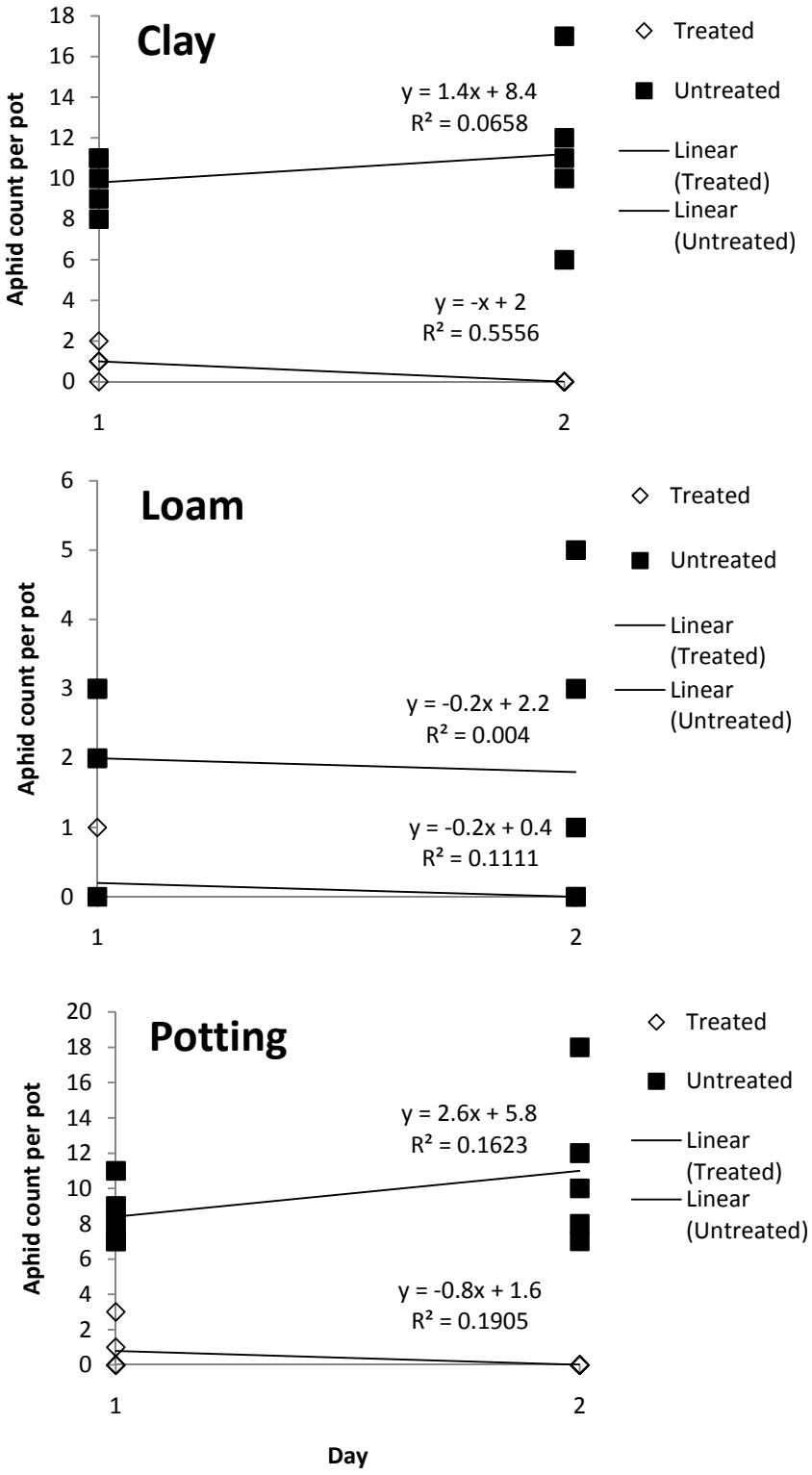

Loam
Aphid count per pot
y = 1.02x + 2.5
R² = 0.1334
y = -0.34x + 1.2
R² = 0.338
Treated
Untreated
Linear (Treated)
Linear (Untreated)


Potting
Aphid count per pot
y = 5.64x - 2.9
R² = 0.7369
R² = 0.5755
Treated
Untreated
Linear (Treated)
Linear (Untreated)
Day

1c. Day 10.


Clay
Aphid count per pot
y = 1.9x + 7.3333
R² = 0.0659
y = -1.4x + 4
R² = 0.5385
Treated
Untreated
Linear (Treated)
Linear (Untreated)
Loam
Aphid count per pot
y = -1.5x + 6.8667
y = -0.6x + 1.6
R² = 0.6429
Treated
Untreated
Linear (Treated)
Linear (Untreated)
Potting
Aphid count per pot
y = 3.6x + 5.6667
R² = 0.1601
y = -0.6x + 1.6667
R² = 0.4655
Day
Treated
Untreated
Linear (Treated)
Linear (Untreated)

1d. Day 12.


Clay
y = 1.4x + 8.4
R² = 0.0658
y = -x + 2
R² = 0.5556
Aphid count per pot
Treated
Untreated
Linear (Treated)
Linear (Untreated)
Loam
y = -0.2x + 2.2
R² = 0.004
y = -0.2x + 0.4
R² = 0.1111
Potting
y = 2.6x + 5.8
R² = 0.1623
y = -0.8x + 1.6
R² = 0.1905
Day